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Inheritance of Seed Zinc Accumulation in Navy Bean

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ABSTRACT

Human zinc (Zn) deficiency is a widespread condition prevalent in people consuming grain and legume based diets. Dry beans (*Phaseolus vulgaris* L.) are frequently the major protein source in such diets. One way to reduce the incidence of Zn deficiency may be through the development of high Zn dry beans. Large variation for dry bean seed Zn concentration exists, which would aid in the development of Zn-rich cultivars. The objectives of this study were to determine the inheritance of seed Zn levels in navy bean and to measure seed phytic acid (PA) levels in relationship to seed Zn concentration as an indicator of Zn bioavailability. A high seed Zn cultivar 'Voyager' and a low seed Zn cultivar 'Albion' were used to create the F_2 and backcross populations that were field grown in 1999 and 2000. Seed Zn was measured in both years and seed phytic acid was measured in 1999. The results of this experiment suggest that a single dominant gene controls the high seed Zn concentration in the Voyager/Albion cross. In addition, phytic acid levels between the parent cultivars used in this study showed little variability and there was no strong correlation between seed Zn and PA concentrations. The development of dry bean cultivars with increased seed Zn levels should be possible through breeding.

Dry bean is a major source of protein and several vitamins and minerals for people in Latin America and Africa. The average per capita bean consumption was 9.9 kg per year in Latin America and 2.7 kg per year in Africa in 2001, with high-end consumption at 27.7 kg per year in Rwanda (FAO, 2003). As compared with meat-based diets, plant-based diets are often limited in the total content and bioavailability of Zn, an essential micronutrient in human nutrition. In fact, marginal Zn deficiency is widespread in people who maintain diets rich in legumes (Torre et al., 1991). An estimated 49% of the world population is at risk for low Zn intake (Brown and Peerson, 2001; International Zinc Association, 2000). The areas of the world where people are most at risk are Latin America, Sub-Saharan Africa, the Caribbean, and Southeast Asia. In these regions, only 15 to 25% of the Zn is from animal sources as compared with developed nations, where more than half of the Zn comes from animal sources (Ferguson et al., 1989).

Phytate, the principle form of phosphorus in legume seeds, is largely responsible for reduced Zn bioavailability (Turnlund et al., 1984). Numerous studies have shown a correlation between high phytate diets and limited Zn absorbance in the gastrointestinal tract of humans and animals (Saha et al., 1994; House et al., 1982; Turnlund et al., 1984; Lonnerdal et al., 1989; Hunt et al., 1998; Zhou et al., 1992). The recommended daily allowance of Zn established for people of the USA is 12 to 15 mg for adults and 10 mg for children. However, this amount of Zn may be too low for people whose diets are vegetarian and phytate rich. Dietary phytate: Zn molar ratios are a major factor in determining the risk of Zn deficiency (Frossard et al., 2000).

One way to alleviate Zn deficiency in humans maintaining legume-rich diets, as seen in countries of Latin America, Africa, and to a lesser extent, vegetarians in the USA is to introduce cultivars with increased levels of seed Zn. In dry bean, this goal may be achieved by screening genotypes for seed Zn concentration. House et al. (2002) found variability for seed Zn from 26.7 to 62.4 $\mu g\ g^{-1}$ dry weight among 18 genotypes of various dry bean market classes. A concern with increasing dietary seed Zn through plant breeding is that the presence of phytate in the seed will negate the effect of increased seed Zn. In a recent study, Donangelo et al. (2003) looked at Zn absorption in young women fed diets formulated from dry bean genotypes with different Zn levels. Although seed Zn content among the genotypes varied by two fold, phytate levels were similar in all genotypes. The women who consumed a high-Zn bean diet compared with a low-Zn bean diet showed an increase in total Zn absorption by 40%, suggesting that breeding for increased seed Zn in bean may, indeed, be a sustainable method to reduce human Zn deficiency.

Knowledge of the inheritance of seed Zn concentration in dry bean is essential to benefit from the potential health benefits of biofortification of this micronutrient essential to human diets. Accordingly, a study was conducted with three objectives: (i) ascertain the inheritance of seed Zn concentration in navy bean, (ii) determine the broad and narrow sense heritability of seed Zn levels, and (iii) measure total seed phosphorus and phytic acid levels to serve as an indication of variability in Zn bioavailability based on phytate presence.

MATERIALS AND METHODS

Navy bean cultivars Albion (Asgrow Seed Company, Kalamazoo, MI, 1987) with low seed Zn and Voyager (Rogers Seed Company, Nampa, ID, 1995) with high seed Zn were used to study the inheritance of seed Zn concentration. Albion also exhibits foliar Zn deficiency symptoms when grown in low to moderate levels of soil Zn. On the basis of this susceptibility, Albion is classified as Zn inefficient. Moraghan and Grafton (1999) discovered that the Zn inefficiency was associated with low levels of seed Zn, although all instances of seed Zn variability seen in dry beans are not necessarily associated with Zn efficiency or inefficiency.

Voyager and Albion were cross-fertilized to produce the

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F_1, F_2, and backcross generations. In 1999 and 2000, Albion (Parent 1), Voyager (Parent 2), F_1, F_2, BC_1P_1, and BC_1P_2 were hand-sown in a field near Erie, ND. The soil type was an Eckman coarse-silty, mixed, superactive, frigid Calcic Hapludolls. Diethylenetrinitrilopentaacetic acid (DTPA), Zn, and Fe and $NaHCO_3$–P concentrations in the soil, which estimate plant-available fractions of these elements, are presented in Table 1. Thirty seeds were distributed uniformly in 1.5-m rows, spaced 0.76 m apart. One row for each parent, the F_1, BC_1P_1, and BC_1P_2, and eight rows for F_2 were planted. The population size evaluated was based on seed availability. Plants were harvested individually by hand and kept separate by placing each plant in a paper bag.

Fertilizer and herbicide applications, and common cultural practices followed recommended practices for dry bean production in the northern Great Plains. Plots were hand weeded when necessary.

Seed from each plant was divided into three subsamples and analyzed for total phosphorus, phytic acid, and Zn concentration.

Total P was determined for one subsample by a molybdenum blue procedure (Murphy and Riley, 1963). Seed was ashed in a muffle furnace at 500°C for 6 h. The ashed samples were acidified with HNO_3 and then diluted 10:1 v:v in 0.3 *M* NaOH. Following the initial dilution, samples were again diluted 15:1 v:v with 1.25 *M* sulfuric acid containing 0.006% ammonium molybdate, 0.0002% antimony potassium tartrate, and 0.005% l-ascorbic acid. The sample absorbance was read on a Brinkmann dipping probe with an 880-nm filter.

A second subsample of seed was used for phytic acid determinations. The PA analysis was based on the methods of Graf and Dintzis (1982) and Sandberg and Ahderinne (1986) and is described below. Phytic acid was extracted from the samples by the addition of 0.5 *M* HCl (trace element grade) to freeze-dried and ground samples. The acidified samples were mechanically agitated for 2 h at 21°C followed by centrifugation at 12 000 g for 15 min. The supernatant was collected and diluted 1:5 (v/v) with distilled, deionized water. The diluted sample was passed through a Bond Elut strong anion exchange column (Varian, Walnut Creek, CA) for purification. The column was washed once with 0.05 *M* HCl, and phytic acid was eluted with 2 *M* HCl. The eluted fraction containing PA was air dried and dissolved in 5mM sodium acetate. The dissolved sample was filtered through a 2-μm filter. Phytic acid levels in each sample were quantified by high performance liquid chromatography. The column used for analysis was a Waters Symmetry C18 column (3.9 × 150 mm) (Waters, Milford, MA) heated to 40°C. Sodium acetate (5 m*M*) was used as the solvent at a flow rate of 1.4 mL min^{-1}. Phytic acid dodecasodium salt from corn (*Zea mays* L.) (Sigma, St. Louis, MO) was used as a standard to determine PA concentration.

The third subsample of seed was used for Zn analysis. Seed was washed with deionized water, oven dried at 70°C for 48 h, and ground with a mortar and pestle to pass through a 0.25-mm mesh sieve. Samples were digested on an aluminum block with HNO_3 and $HClO_4$. Seed Zn concentration was determined by atomic absorption spectroscopy. Standard Reference Material 1572 and 1515 from the National Institutes of Standards and Technology, Gaithersburg, MD, and an acid blank were digested and analyzed concurrently with samples.

Chi-square analyses (χ^2) were conducted for frequency distributions of seed Zn in the F_1, F_2, and backcross generations to test the goodness of fit of the data to hypothesized genetic ratios. Discriminant analysis was the statistical procedure used to classify each plant in the F_1, F_2, and backcross generations into either high or low seed Zn genotype. In this procedure, the pooled standard deviation of Voyager and Albion was estimated. The cutoff value between the high and low Zn classes was determined by subtracting the mean of Albion from the mean of Voyager. The resulting number was multiplied by the mean of Albion plus the mean of Voyager and divided by two. Any sample with a value above the resulting number was categorized as high seed Zn, and any sample with a value below the number was categorized as low seed Zn genotype.

Table 1. Average soil content of Zn, Fe, P, and soil pH at Erie, ND during the 1999 and 2000 growing seasons.

Year	pH	DTPA-Zn†	DTPA-Fe	$NaHCO_3$–P
		mg kg^{-1}		
1999	5.8	2.4	84	21
2000	7.2	1.0	31	17

† DTPA = diethylenetrinitrilopentaacetic acid.
All values represent samples from a depth of 0 to 152 mm.

A mixed effects model (PROC MIXED in SAS, 1997) was used to determine significant variation among means of the six generations for seed Zn, P, and PA. In this model, replications of measurements on the seed of a single plant were considered random effects, and generations were considered fixed effects.

Generation means analysis was also used to elucidate the inheritance of Zn and PA in the seed. Weighted least squares regression was used to fit six generations to six variables beginning with the midparent. Each generation was weighted by the inverse of the variance of its mean (Mather and Jinks, 1971). The six variables used in the model to describe the phenotype were midparent value (m), additive effects [d], dominance effects [h], additive × additive interactions [i], additive × dominance interactions [j], and dominance × dominance interactions [l]. The model parameters were estimated from the means of each generation and genetic coefficients (Holthaus et al., 1996). Relationships among the six generations used to establish gene effect estimates for generation means analysis (Gamble, 1962) were $m = F_2$, $[d] = P_1F_1 - P_2F_1$, $[h] = -0.5P_1 - 0.5\,P_2 + F_1 - 4F_2 + 2P_1F_1 + 2\,P_2F_1$, $[i] = 4F_2 + 2P_1F_1 + 2P_2F_1$, $[j] = -0.5P_1 + 0.5P_2 + P_1F_1 - P_2F_1$, $[l] = P_1 + P_2 + 2F_1 + 4F_2 - 4P_1F_1 - 4P_2F_1$. From the above estimates, expected means were found and a chi square test was used to determine if the data fit an additive/dominance model which only included m, [d], and [h] or an epistasis model which takes all six gene effects into consideration. We used *t* tests to determine which of the genetic estimates were significant. These analyses were conducted with the aid of a statistical spreadsheet developed by Ng (1990).

Estimates of broad sense and narrow sense heritability were calculated for seed Zn concentration by using the variance of the parent, F_1, F_2, and backcross generations to estimate phenotypic (V_P), environmental (V_E), total genetic (V_G), additive genetic (V_A), and dominance genetic variances (V_D). Where:

$$V_P = V_{F2}$$
$$V_E = 0.25(V_{P1}) + 0.25(V_{P2}) + 0.5(V_{F1}),$$
$$V_G = V_{F2} - V_E,\ V_A = 2(V_{F2}) - V_{BC1P1} - V_{BC1P2}$$
$$V_D = V_{BC1P1} + V_{BC1P2} - V_{F2} - V_E.$$

Broad sense heritability = $h^2_b = (V_A + V_D)/V_{F2}$, where $V_A + V_D$ represent the genetic variance of F_2 (Allard, 1960). Narrow sense heritability = $h^2_n = V_A/V_{F2}$ (Warner, 1952).

Table 2. Mean levels of total seed phosphorus, phytic acid, the phytate Zn molar ratio, and seed Zn in two navy bean cultivars and four additional generations established by an initial cross between the cultivars from 1999 seed.

Generation	1999 Phosphorus	1999 Phytic acid	1999 Phytate Zn molar ratio	1999 Zinc	2000 Zinc
	mg g^{-1}			mg kg^{-1}	
P_1 (Albion)	4.9a	6.0a	35a	21.7a	14.4a
P_2 (Voyager)	4.9a	7.1a,b	42a,b	31.2b	21.7b
F_1	5.0a	7.8b	45b	28.5c,d,e	23.1b
F_2	4.9a	8.9c	52c	28.5c	22.6b
BCP_1	4.9a	8.9c	52c	26.5e	16.6c
BCP_2	4.3b	9.9d	64d	30.7b,d	19.8d

Means that do not share a letter are significantly different from each other (Tukey's test, $P < 0.05$).

RESULTS AND DISCUSSION

Seed Zinc Concentration

Seed Zn concentration was higher in Voyager than Albion for both years, and the total seed Zn concentration of each cultivar was higher in 1999 than 2000 (Table 2). The difference in mean seed Zn concentration between years was probably due to variability in the soil DTPA-Zn. The DTPA soil Zn was 2.4 and 1.0 mg kg^{-1} in 1999 and 2000, respectively. The soil pH also may have contributed to the differences. The soil pH at Erie, ND, was 5.8 and 7.2 in 1999 and 2000, respectively. Soil pH above 7.0 may have reduced Zn availability for plant growth because of the affinity for Zn to bind

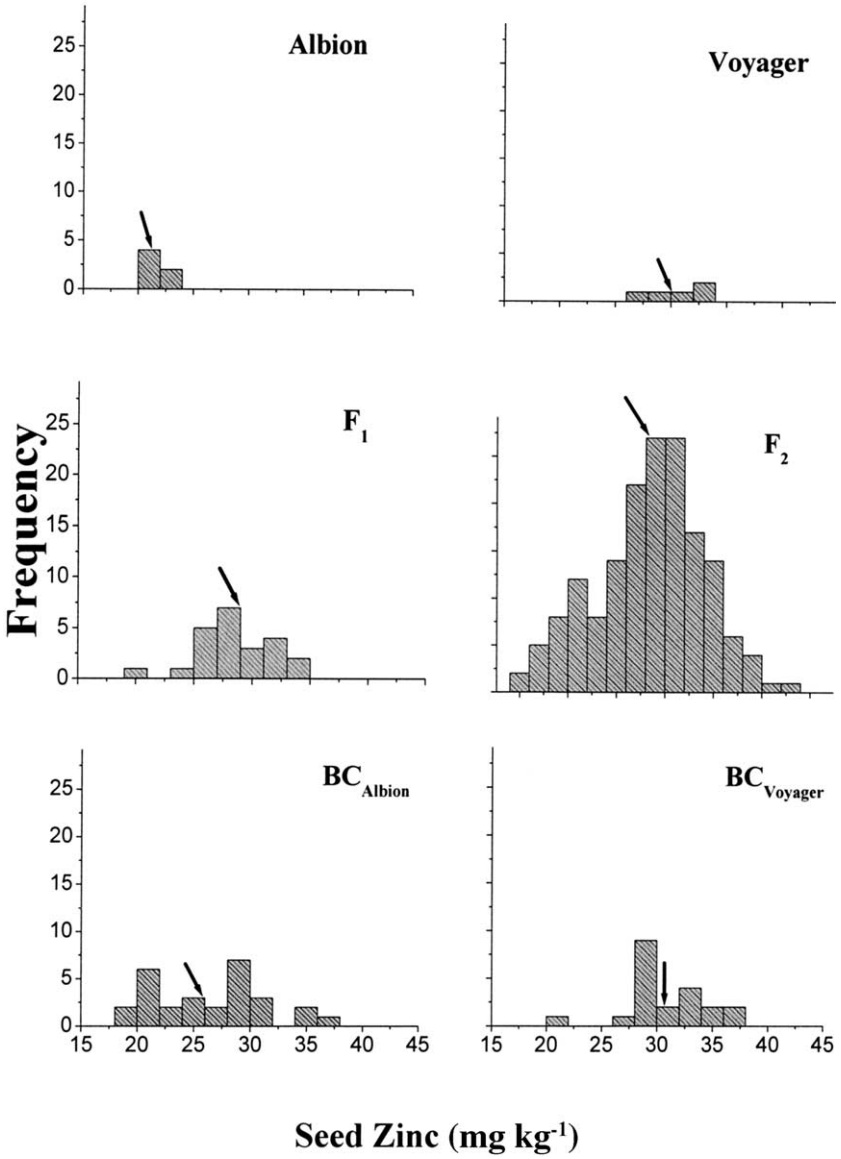

Fig. 1. Frequency distribution of Zn concentration in Albion and Voyager navy bean seed and four generations derived from the intermating of Albion and Voyager in the 1999 field season. The arrows indicate the mean Zn concentration of each generation.

with clay or $CaCO_3$ at higher pH (Marschner, 1997). In 1999, no visual foliar Zn-deficiency symptoms were observed. In 2000, Albion and some F_2 plants exhibited characteristic Zn deficiency symptoms. The observation that Voyager contained higher levels of seed Zn than Albion in both years in soil conditions differing dramatically in their plant available Zn fraction (Table 1) indicated the potential value of locating high seed Zn concentration plants across environments.

Seed from each plant of the F_1, F_2, and the two backcrosses derived from Albion × Voyager grown in 1999 and 2000 were scored for high or low seed Zn concentration. In 1999, the F_2 plants ranged in Zn concentration from 15.4 to 43.7 mg kg^{-1}. Upon classification, the population consisted of 117 high seed Zn and 51 low seed Zn plants (Fig. 1), giving a good fit to 3 high: 1 low seed Zn (Table 3). The BC_1P_1 population fit a 1 high: 1 low seed Zn ratio. The BC_1P_2 consisted of 20 high Zn plants and 1 low Zn plant. The 1999 data support our conclusion that high seed Zn was controlled by a single dominant gene.

In 2000, the F_2 progeny ranged in Zn concentration from 12.4 to 34.2 mg kg^{-1}. The mean of the F_2 progeny was higher than the mean of Voyager. The transgressive segregation observed may be the result of soil variability or the effect of minor gene(s). The seed Zn distribution in the F_2 was 16 low Zn to 77 high Zn plants and resulted in a chi-square value of 3.01 ($P < 0.08$) (Table 3), which precludes making a definitive conclusion as to whether the trait fits the expected 3:1 ratio for a single gene. The mean seed Zn concentration of BC_1P_1 progeny was 16.6 mg kg^{-1} and intermediate to the means of Voyager (21.7 mg kg^{-1}) and Albion (14.4 mg kg^{-1}). The mean seed Zn concentration of BC_1P_2 progeny was 19.8 mg kg^{-1}. The distribution of 9 low seed Zn concentration to 8 high seed Zn concentration for the BC_1P_1 progeny gave the expected 1:1 ratio for a trait controlled by a single dominant gene. The frequency distribution of seed Zn concentration on a per plant basis for each generation is shown in Fig. 2.

Table 3. Chi-square tests for a one-gene model for the genetic control of the seed-Zn accumulation trait in the F_1, F_2, and backcross generations of Albion/Voyager grown at Erie, ND, in 1999 and 2000.

Population	Seed Zn	Expected	Observed	χ^2	$P <$
1999					
F_1	high	23	21		
	low	0	2		
	total	23	23	0.004	0.14
F_2	high	126	117		
	low	42	51		
	total	168	168	3.64	0.11
BC_1P_1†	high	14.5	15		
	low	14.5	14		
	total	29	29	0.002	0.85
BC_1P_2‡	high	21	20		
	low	0	1		
	total	21	21	0.002	0.91
2000					
F_1	high	24	24		
	low	0	0		
	total	24	24	0.00	0.995
F_2	high	69.75	77		
	low	23.25	16		
	total	93	93	3.01	0.08
BC_1P_1	high	8.5	8		
	low	8.5	9		
	total	17	17	0.06	0.81
BC_1P_2	high	26	26		
	low	0	0		
	total	26	26	0.00	0.995

† P_1 (Albion).
‡ P_2 (Voyager).

The chi-square analyses demonstrated that a single gene controls seed Zn in this navy bean cross and that high seed Zn is dominant over low seed Zn. Singh and Westermann (2002) reported that a single dominant gene controls the Zn efficiency trait in a cross between Matterhorn (Zn efficient) and T-39 (Zn inefficient). Unlike our work, Singh and Westermann's (2002) findings were based on field observation of foliar Zn deficiency symptoms. It is tempting to speculate that the same gene influences both foliar Zn and seed Zn content in bean. Conversely, Hacisalihoglu et al. (2004) found Zn efficiency was not correlated with seed Zn concentration in 35 bean lines tested and suggested Zn efficiency is under separate genetic control than seed Zn content and concentration.

The chi square tests indicated a single gene controlled seed Zn concentration in the Voyager × Albion population in 1999 and 2000 (Table 3); however, the apparently normal distribution of the F_2 generation in 1999 (Fig. 1) and the borderline fit to a single gene ratio in 2000 (Table 3) suggested generation means analysis would be a useful method to determine gene action for seed Zn concentration. In 1999, the midparent value of seed Zn was 25.6 mg kg^{-1}, and the mean value of the F_1 population was 28.5 mg kg^{-1}. A linear contrast (data not shown) indicated that the F_1 value was significantly higher than that of the midparent ($p = 0.05$), which suggested that the gene controlling Zn efficiency has partial dominance. However, examination of the genetic effects from the F_2 and backcross generations (Table 4) revealed that only the m and [d] parameters were significant, thus, indicating that additive gene effects were responsible for the majority of the variation for seed Zn. The chi-square test for the adequacy of an additive–dominance model for Zn concentration indicated that the model was nondisturbed by epistatic interactions. In 2000, generation means analysis (Table 4) indicated a role for additive and dominance main effects and additive × additive and dominance × dominance epistatic interactions in the inheritance of seed Zn concentration. The differences in the results of generation means analysis in 1999 and 2000 indicate a genotype × environment interaction. Although in both years Voyager had higher levels of seed Zn than Albion, only in 2000 did Albion exhibit foliar Zn deficiency symptoms. The decreased levels of Zn in the soil available to the plant may have activated additional genes involved in Zn accumulation in the seed that were not evident in the 1999 field season.

Broad and narrow sense heritabilities for seed Zn in the 1999 field season were 0.84 and 0.82, respectively, and broad sense heritability was 0.85 in 2000. The high narrow sense heritability value for seed Zn is consistent with the conclusion that additive genetic variance is a major component of the total genetic variance arising from the locus controlling seed Zn. The high values of

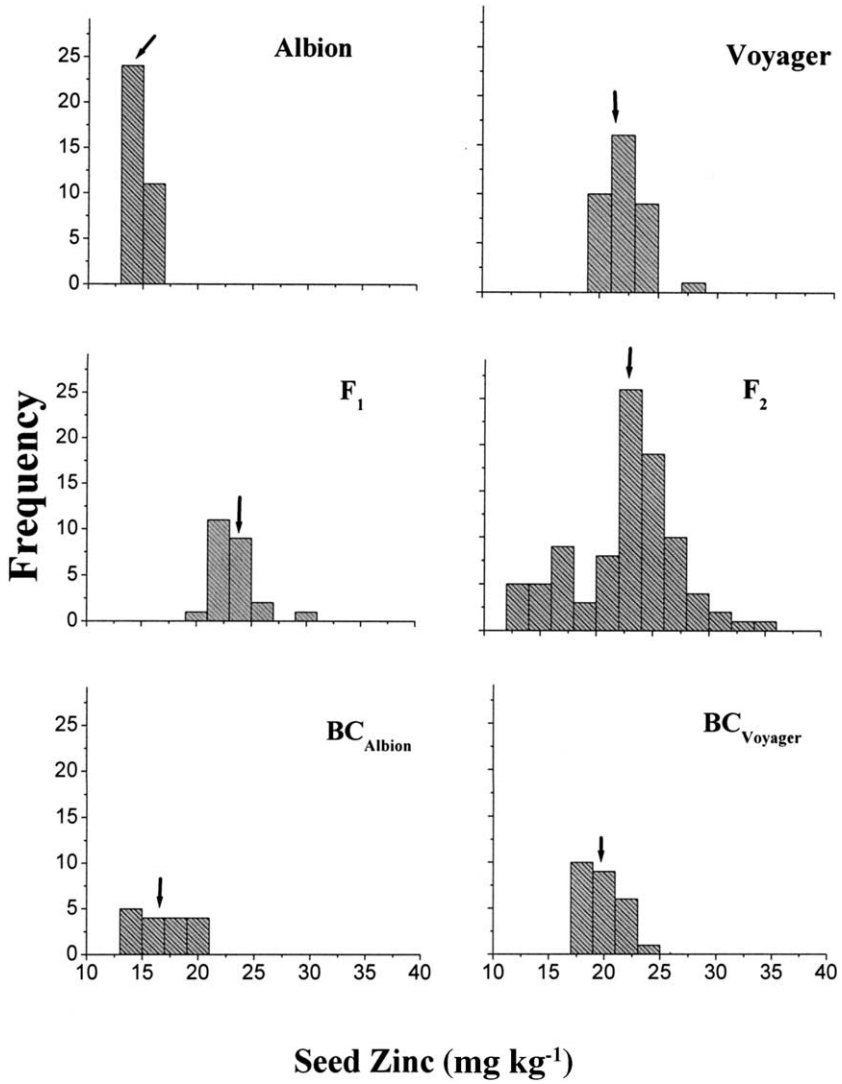

Fig. 2. Frequency distribution of Zn concentration in Albion and Voyager navy bean seed and four generations derived from the intermating of Albion and Voyager in the 2000 field season. The arrows indicate the mean Zn concentration of each generation.

broad and narrow sense heritability estimates for seed Zn indicated that selection for increased levels of seed Zn would be effective in early generations especially in soils with high available Zn content. Selection could begin in the F_2 and a pedigree, backcross or single seed descent breeding strategy would be useful to develop lines with increased levels of seed Zn.

The high heritability of this trait should be confirmed in other market classes and breeding populations of dry bean. A recent study by Guzman-Maldonado et al. (2003) found a single QTL explaining 15.2% of the variability for seed Zn concentration in a cross between the beige seed coat colored cultivar, Baranda, and a wild black seeded genotype, G22837. On the basis of the high heritability found for seed Zn concentration in our study, one would expect that Guzman-Maldonado et al. should have seen a higher percentage variability than what they reported. *Phaseolus vulgaris* is classified into two gene pools on the basis of seed protein differences (Gepts et al., 1986). The gene pools, Andean

Table 4. Estimates and standard error of genetic effects in the generation means analysis model for the concentration of seed phytic acid phosphorus and Zn in a cross between Voyager and Albion navy bean cultivars from the 1999 and 2000 field seasons with genetic effects: midparent value (m), additive effects [d], dominance effects [h], additive × additive interactions [i], additive × dominance interactions [j], and dominance × dominance interactions [l].

	Genetic Effects ± SE					
Seed trait	**m**	**[d]**	**[h]**	**[i]**	**[j]**	**[l]**
PA (1999)	**1.0 ± 0.2****	**0.16 ± 0.11**	**4.6 ± 0.6****	**0.82 ± 0.18****	**0.26 ± 0.25**	**−3.4 ± 0.4****
Zn (1999)	**26 ± 3.0****	**4.7 ± 0.5****	**8.1 ± 8.2**	**0.7 ± 3.0**	**−1.2 ± 2.6**	**−5.3 ± 5.4**
Zn (2000)	**36 ± 2.3****	**3.7 ± 0.2****	**−41 ± 5.5****	**−18 ± 2.3****	**−1.1 ± 1.4**	**28 ± 3.4****

*** Significance at the 0.05 probability level on the basis of *t* tests with $n - 1 = 5$ degrees of freedom.**
**** Significance at the 0.01 probability level on the basis of *t* tests with $n - 1 = 5$ degrees of freedom.**

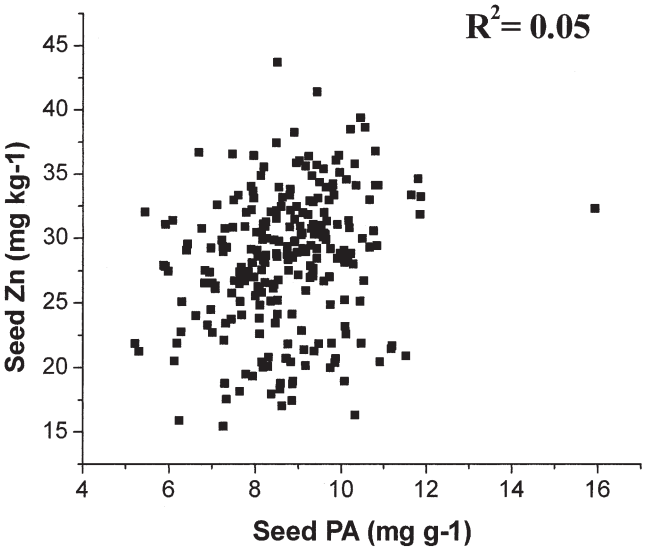

Fig. 3. Correlation between seed Zn concentration (mg kg^{-1}) and seed PA concentration (mg g^{-1}) in the 1999 field season.

and Mesoamerican, differ in genetic architecture–dwarf lethal (DL) genes are an example of genetic differences (Koinange and Gepts, 1992). Guzman-Maldonado studied a population that resulted from an Andean (Baranda) by a Mesoamerican (G22837) cross. The low variability of seed Zn concentration seen in their study as compared with our work may have resulted because of differences in the genetic architecture between the two gene pools.

Seed Phytic Acid Concentration

Total phosphorus and phytic acid seed levels were measured in 1999 alone. The only variability for seed total P was observed in the BC_1P_2 (Table 2). There was no difference between Albion and Voyager for PA concentration of seed. The mean values for this trait were 6.0 and 7.1 mg g^{-1} for Albion and Voyager, respectively (Table 2). Since the mean values of PA of Albion and Voyager were similar, we were not able to categorize the generations into high and low PA classes. However, the generation means analysis provided information for which some genetic inferences could be made. On the basis of linear contrasts, the mean of the F_1 generation (7.8 mg g^{-1}) deviated significantly from the mid-parent value (6.6 mg g^{-1}). The significant deviation of the F_1 from the mid-parent indicated that in this population dominance was a property of the genetic system influencing phytic acid content in bean seeds.

There was not a strong correlation (R^2 = 0.05) between PA and Zn levels in the Voyager × Albion population tested (Fig. 3), which suggests breeding for increased seed Zn will not also increase seed PA. Although PA was not different between the parents used in this study, there were higher levels of PA in the F_2 and backcross generations than either parent as well as higher Zn: PA molar ratios (Table 2). Welch et al. (2000) found variability in phytic acid levels in 24 dry bean lines tested ranging from 19.57 to 29.16 μmol g^{-1}). These data suggest a need for screening for seed PA concentration when breeding for enhanced seed Zn.

Variability in dry bean seed Zn concentration has an influence on the amount of Zn absorbed by the human body on consumption of beans (Donangelo et al., 2003). The potential to use this variability to benefit human health can be realized through plant breeding. In the Albion × Voyager population used in the current study, seed Zn concentration was simply inherited and highly heritable across environments. Therefore, selection for increased seed Zn concentration should be relatively rapid and straightforward to achieve. In addition to genetic manipulation of beans for high seed Zn concentration, improved Zn fertility of the soil may also increase seed Zn concentration. We also recommend screening PA levels in bean lines with high seed Zn concentration to ensure that increased levels of phytate do not negate the value of gains in Zn in the diet.

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